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(54) Title: SYSTEM FOR PROCESSING AND IMAGING OF SAMPLES

(57) Abstract: The invention features a system for processing a sample, including: (a) a container for storing and transporting the sample; (b) means for infiltrating and embedding the sample to form a block in the container; (c) means for sectioning the sample in the block; (d) means for imaging the sample in the block; and (e) means for identifying the sample during the processing of the sample.

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SYSTEM FOR PROCESSING AND IMAGING OF SAMPLES

Background of the Invention

The invention relates to the field of histology.

The processing of organic tissue samples and other materials for transmission microscopy, both visible light and electron microscopy, is normally carried out by subjecting the sample to a series of chemical treatments culminating in the production of a solid block in which the sample is embedded.

In conventional processing, the tissue sample is first chemically fixed with formalin, glutaraldehyde, or other material which serves to preserve the sample from autolysis (self-degradation), to render the sample rigid, and to increase its permeability, thereby enhancing the infiltration of the subsequent solutions. The infiltration steps which follow chemical fixing remove all of the water from the sample through progressive replacement of water with increasing concentrations of solvents such as alcohol and xylene. Infiltration is followed, for example, by treatment with melted paraffin, and the sample then is cooled to room temperature whereupon it solidifies. Alternatively, the tissue is infiltrated with plastic polymer that is then hardened by heat, ultraviolet light or other means. The hardened, infiltrated tissue is then positioned in a mold and surrounded with paraffin or plastic to produce a tissue block.

In conventional tissue processing methods for standard light and electron microscopy, thin sections of the sample are cut from the block and transferred to glass slides or some other support. The tissue is stained to improve image contrast and then viewed under the microscope to give a two-dimensional (XY) image. Information about three-dimensional structures in the tissue is obtained by examining successive slices of the tissue.

United States Patent No. 4,960,330 (hereby incorporated by reference) describes a block sectioning and image acquisition system in which successive sections are removed from the block and the emerging block faces are imaged using either a microscope or a scanning laser. The method provides rapid recording and storage of structure information without the time-consuming and error-prone handling of individual tissue sections. Surface imaging offers the advantage that three-dimensional reconstruction, as well as generation of XZ and YZ two dimensional images, can be readily obtained.

Summary of the Invention

The present invention allows for the consolidation and simplification of the processing and imaging of tissue samples. In the system of the invention, clinicians and scientists (the clients) send tissue samples to a processing center and later receive hardcopy or digital images of the sectioned samples. In this system, the client can modify the digital images, or even produce new digital images from the data (i.e., the sum of digital images) provided by the processing center.

Accordingly, in a first aspect, the invention features a system for processing a sample, including: (a) a container for storing and transporting the sample; (b) means for infiltrating and embedding the sample to form a block in the container; (c) means for sectioning the sample in the block; (d) means for imaging the sample in the block; and (e) means for identifying the sample during its processing.

The system of the invention can include means for producing and storing a digital image of the sample, as well as a computer program for visualizing the digital image. The system can also include means for chemically fixing the sample prior to infiltrating and embedding.

In a second aspect, the invention features a method of imaging a sample. The method includes: (a) providing a container suitable to hold a sample; (b) chemically fixing the sample in the container; (c) infiltrating and embedding the sample to form a

block in the container; (d) sectioning the sample in the block; and
(e) producing an image of the sample in the block.

It is preferred that the method includes step (f), storing the image as a digital image, as well as step (g), visualizing the digital image.

By "sample" is meant tissue or other material which is to be stained and embedded. The thickness of a sample can be greater than 200 microns, one millimeter, one centimeter, or more.

By "fixation" is meant the treatment of tissue specimens with a chemical solution that preserves, hardens, and permeabilizes the sample.

By "infiltration" is meant treating the tissue with a liquid or series of liquids which penetrate throughout the tissue to the molecular level and are then transformed into a solid in order to render the sample rigid.

By "embedding" or "embedment" is meant positioning the infiltrated tissue in a mold and surrounding it with a substance (usually the same as the infiltrating substance) which is then hardened to form an encasing block. The embedding substance thus serves to provide rigid support and to facilitate the cutting process.

By "sectioning" is meant cutting from the block thin slices.

By "staining" is meant treating a material with a colored or fluorescent substance that associates with the material on the molecular level.

"Fluorescence" or "darkfield" staining is accomplished using unconjugated dyes (i.e., dyes that are naturally fluorescent when excited with light of the proper wavelength) or conjugated dyes (i.e., molecules that bind to the sample and that are attached, either directly or indirectly, to a fluorochrome). Examples of conjugated dyes include without limitation: (i) a primary antibody that binds to an antigen and a secondary antibody, containing a fluorochrome, that binds to the primary antibody; and (ii) a molecule that has, covalently bound to it, a fluorochrome. Fluorescence staining results in images that have a black background, while "standard" or

"brightfield" staining is accomplished using dyes that are usually non-fluorescent and results in images with a bright, or white background. It is understood that the same dye could be useful for both fluorescence and standard microscopy.

Surface imaging offers the advantage that sectioning and imaging can be, to a large degree, automated. The reduction in human effort, combined with the savings in mounting materials, reduces the cost or, alternatively, provides more data for the same cost. The invention provides a method for the accumulation of more data (in the form of digital images) without an increase in material and labor costs.

Other features and advantages of the present invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description of the Invention

We have discovered a system for processing and imaging of a tissue sample. By virtue of the use of (i) a single container, labeled for identification purposes and compatible with every step of the processing of the sample; and (ii) a block sectioning and image acquisition system, the invention provides highly automated tissue processing and imaging.

The system of the present invention includes the following general components: a container for storing and transporting the sample; means for infiltrating and embedding the sample to form a block in the container; means for sectioning the sample in the block; means for imaging the sample in the block; and means for identifying the sample during the processing of the sample. Preferably, the system also includes means for chemically fixing the sample prior to infiltrating and embedding, although this is not essential. In particular preferred embodiments, the system also includes: means for producing and storing a digital image of the sample; and a computer program for visualizing the digital image.

The system of the invention, and methods for its use, is particularly compatible with block face microscopy. Block face microscopy is advantageous over standard

brightfield microscopy in that block face methods allow for the generation of high-quality microscopy images of biological tissue and other materials without the need to manufacture glass histology slides. The elimination of this requirement permits full automation of the histopathologic process, reducing incremental costs for each additional section produced, and consequently allowing for much greater amounts of information to be collected from each sample. Additionally, the images are readily stored in digital format, facilitating computer aided analysis of the images, such as measurement of area or volume, or three dimensional reconstruction.

In block face microscopy, the digital "virtual" section, as captured unmodified from the block face, is a dark field image resulting from the colored emissions from the fluorescence-stained sample appearing against a black background representing the opacified polymer in which the sample is infiltrated and embedded. In contrast, conventional optical transmission microscopy, including that practiced in most surgical pathology laboratories and other medically-related microscopy-based diagnostic facilities, produces a brightfield image because thin slices of tissue and other material are stained with standard non-fluorescent dyes and are then trans-illuminated with a white or near-white light source, resulting in a background that is brighter, rather than darker than the tissue image. The raw darkfield images captured from the face of the block can be transformed and displayed as the more familiar images encountered in brightfield microscopy. Methods for such image transformation are described, for example, in U.S.S.N. 09/311,789, which is hereby incorporated by reference.

Description of the Components

The container

The tissue sample of interest is placed in a container that, preferably, is compatible with all steps of tissue processing, including storing, transporting, chemically fixing, infiltrating, embedding, sectioning, and imaging. For example, the container preferably serves as a mold for use in the preparation of a sample to be

sectioned and examined. Thus, the mold will define the shape of the block in which the tissue sample is embedded. In preferred embodiments, the mold defines an elongated cavity, including an opening at a proximate end for introduction of a tissue sample from outside the mold. The proximate end defines a shape adapted for engagement with a securing means. The mold tapers to a distal end which defines a polyhedron of a size and shape suitable for sectioning; this polyhedron is referred to as the specimen chamber. The shape of the mold should be compatible with the size and shape of both the imaging field and the tissue sample. Thus, the distal end of the mold preferably approximates the size and shape of an imaging field of an imaging apparatus which may be used to view an emerging face of a tissue block prepared from the mold, such that the cross-section of the specimen chamber transverse to the length of the block, or the exposed surface of the sample chamber, approximates or is of similar size and shape as the imaging field of an imaging apparatus used to view the surface. The imaging field is the area over which an imaging device can record data which relates to the surface. It is desired that the exposed face of the block which is to be viewed be neither significantly larger nor smaller than the imaging field. The imaging device provides microscopic imaging of the surface, that is, the actual scale of the imaged area is not dictated by the scanning array but rather by the magnification optics. Thus the size of the imaging field is determined by the magnification optics and the shape of the imaging field is dictated by the array design.

It is understood that a suitable container shape is selected based on tissue sample size and optimal imaging geometry. For example, a punch biopsy of the skin may produce a round skin sample of about 4 mm in diameter. A container having a 4.5 mm x 4.5 mm square specimen chamber readily accommodates such a sample and, results in a block with an imaging corresponding substantially to the imaging field.

When the sample is of an irregular shape, the shape of the specimen chamber may be selected accordingly. For example, a prostate needle biopsy produces a sample that is approximately 1 mm in diameter and 17 mm in length. A preferred

container for such a tissue sample would possess a specimen chamber having that shape as well, for example, by having a rectangular cross-section with dimensions of about 2 mm across and 20 mm long. In such cases, a linear CCD scanner may be used to generate a rectangular imaging field.

Although the quality of a section cut from such a long thin block face may be less than optimal for examination with conventional microscopy techniques, the quality of the exposed, cut face is unaffected. Thus, when the molded block is used in conjunction with the automated image recording apparatus of U.S. Patent No. 4,960,330, a wide variety of shapes of the container, not heretofore considered suitable for tissue imaging, can be employed in order to obtain high quality imaging area.

The container may be prepared from any conventional material, for example, polypropylene, silicone or polystyrene. Selection of the material may be influenced, in part, by the intended use of the container. The container may be reusable or sacrificial. Reusable containers are prepared from flexible materials such as silicone. Sacrificial containers include containers which remain on the molded block during sectioning operations, as well as containers that are destructively removed from the molded block. In the instance where the container is intended to be removed, the sacrificial containers desirably are prepared using materials that fracture or tear easily. Suitable materials include polypropylene. In the instance where the mold is intended to be carried through the cutting process, which is the preferred method, the container desirably is prepared from rigid or sturdy material that lends itself to being cut. Suitable materials include polystyrene.

In preferred embodiments, the container is threaded so that a cap may be used to close the container. Alternatively, snap-on caps or other leak-proof caps may be used.

Means for identifying a sample during processing

The container is preferably labeled so that it can be distinctly identified. There are many means by which a container can be identified as being distinct from all other containers; any of these means can be used in the present invention. In one example, each container is labeled with a unique bar code. Such labeling of containers allows for the identification of a sample during processing of large batches of samples.

Means for chemical fixation, infiltration, and embedment of a sample

A tissue sample is embedded into a molded block using standard embedding methods. Thus, for example, the tissue is first chemically fixed with standard fixative liquids to preserve the sample from autolysis (self-degradation), to render the sample rigid, and to increase its permeability, thereby enhancing the infiltration of the subsequent solutions. Thereafter, the water from the sample is removed through progressive replacement of water with increasing concentrations of solvents such as alcohol and xylene. Infiltration is followed by treatment with melted paraffin or with plastic polymer to produce a hardened infiltrated tissue. The preceding steps are carried out in the container. The hardened, infiltrated tissue is then placed in the specimen chamber of the container and is surrounded with paraffin or plastic to produce a tissue block.

Means for sectioning a sample

A sectioning apparatus typically includes a reciprocating bar supporting a tissue block holder and a knife holder with a blade. The reciprocating bar moves the tissue holder (and the molded block secured thereto) up and down and advances the molded block towards the blade to remove a section of the block. When the molded block is used in conjunction with the automated image recording apparatus described in U.S. Patent No. 4,960,330, which is incorporated in its entirety by reference, the tissue sections cut from the block are discarded or are saved for later use, as described in U.S. Patent No. 5,746,855, hereby incorporated by reference, and the exposed face

of the block is examined. The microtome is positioned on a base which also supports an imaging apparatus for viewing the exposed face of the block.

Means for imaging a sample

Typical imaging apparatuses which can be used in the imaging of the molded block of the invention include area array and linear charge coupled devices (CCDs). A CCD is a detector that provides digital images. The digital format allows the images to be manipulated by a computer, which can electronically modify or copy them. Electronic storage and editing of images provide significant advantages over conventional photographic methods.

The viewing field of an area array scanner is a rectilinear area defined by a two-dimensional array of pixels. The greater the number of pixels defining the image, the greater the resolution. A low resolution area array CCD may have dimensions of 300 x 600 pixels, while higher resolution CCDs have dimensions on the order of 2000 x 2000 pixels. A linear CCD scanner has only one or a few rows of pixels. A complete image is obtained by incrementally advancing the linear CCD (or the sample) as the scanner moves across the width of the sample. In both case, the imaging field defines a rectilinear area. Rarely, non-rectilinear CCDs may be employed.

The selection of the size and shape of the specimen chamber is made with consideration of the type of imaging apparatus used to capture the images from the exposed face of the block. Thus, the specimen chamber shape is selected to avoid empty space in the imaging area or extension of the sample outside the imaging area. These circumstances are to be avoided where possible because they reduce the amount of digital data (pixels) that correspond to the tissue sample. The greater the pixel number defining the sample (as compared to the empty space), the greater the resolution. Thus, by minimizing background in the imaging field, imaging resolution of the sample is improved.

Means for producing and storing a digital image of a sample

The acquired image can be stored in digital format using any available storage medium. A preferred medium is one that can be easily transported, for example by mail, or one that can be sent to the client electronically (e.g., by the internet).

The sample

The system of the invention can be used to process any type of sample, whether biological or non-biological. Examples of biological samples are biopsy and autopsy samples. Examples of non-biological samples are samples from the paper industry (e.g., paper towels), mineral samples from the mining industry, and polymer and plastic samples used in manufacturing (e.g., of automobiles).

The following example is to illustrate the invention, and is not meant to limit the invention in any way.

Example

The client collects a punch biopsy of skin from a patient or animal and places it in a container; labeled with a bar code and containing chemical fixative. Preferably, the container and the fixative are each provided by the processor (the person or company that sections and images the sample). Preferably, the processor also supplies the chemical fixative, which may be supplied in the container or, alternatively, in a second container (and, thus, transferred to the first container by the client). Following placement of the sample in the container, the client places a threaded cap onto the container. The client records the bar code number and the sample contents for later identification, and then sends the sample by appropriate means to the processor.

The processor scans the bar code into the computer that will collect the digital images of the sectioned sample. The container is placed in an automated sample processing machine (e.g., Leica LYNX auto tissue processor; Wetzlar, Germany), and

the sample is processed through a series of processing solutions (e.g., stains, graded alcohols, and/or xylene or other organic solvent), then liquid polymer containing optical additives (e.g., an opacifier).

Prior to hardening of the polymer into a solid block, the sample is placed in the specimen chamber at the tip of the container. This can be easily achieved, for example, by centrifugation. The orientation of the sample relative to the sectioning plane is unimportant, because orientation of the digital image can be performed by a computer during visualization. The polymer is hardened using any appropriate method (e.g., heat, UV, time, etc.)

Once hardened, the sample and the block are mounted on a microtome for sectioning. In one embodiment, the block is left in the container, and the container is sectioned along with the embedded sample. Alternatively, the container can be removed, either entirely or partially (e.g, only the container adjacent the tissue chamber), prior to sectioning. The sample is then sectioned and imaged. Digital images of the sample are stored in a computer. Following sectioning, the block can be discarded or, if unsectioned sample remains in the block, stored for later use. The sections themselves can also be discarded or saved (e.g., for archival purposes). One method for collecting sections is described in U.S. Patent No. 5,746,855.

At all steps of the process, it is desired that the sections, or images of the sample, be labeled with a bar code number corresponding to the one on the sample container. The digital images, appropriately labeled, are sent to the client using a suitable storage medium (e.g., diskette, CD, and the like). In one preferred method, the digital images are transferred to the client electronically (e.g., by the internet). It may be desirable to compress the computer file for greater ease in sending the digital image. It is understood that the format of the digital images is unimportant provided the client has the means to view the images. Thus, it may also be desirable that the processor provide to the client computer software for viewing of the images. Such software may also allow further modifications (e.g, three-dimensional reconstruction, imaging of the XZ or YZ plane, etc.).

Other Embodiments

The system of the present invention may be used in conjunction with any conventional microscopic tissue preparation techniques. For example, the invention may be used in conjunction with the stains and embedding polymers described in co-pending application U.S.S.N. 09/154,430, hereby incorporated in its entirety by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the claims.

What is claimed is:

1. A system for processing a sample, comprising:

(a) a container for storing and transporting said sample;

(b) means for infiltrating and embedding said sample to form a block in said container;

(c) means for sectioning said sample in said block;

(d) means for imaging said sample in said block; and

(e) means for identifying said sample during said processing of said sample.

2. The system of claim 1, wherein said block is in said container when said sample and said block are sectioned.

3. The system of claim 1, wherein said system further comprises means for producing and storing a digital image of said sample.

4. The system of claim 3, wherein said system further comprises a computer program for visualizing said digital image.

5. The system of claim 1, wherein said system further comprises means for chemically fixing said sample prior to infiltrating and embedding.

6. A method of imaging a sample, said method comprising:

(a) providing a container suitable to hold a sample;

(b) chemically fixing said sample in said container;

(c) infiltrating and embedding said sample to form a block in said container;

(d) sectioning said sample in said block; and

(e) producing an image of said sample in said block.

7. The method of claim 6, wherein said block is in said container when said sample and said block are sectioned.

8. The method of claim 6, wherein said method further comprises (f) storing said image as a digital image.

9. The method of claim 8, wherein said method further comprises (g) visualizing said digital image.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/807; 422/99, 102; 382/133

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,991,028 A (CABIB et al) 23 November 1999, see abstract.	1-9
Y	US 4,960,330 A (KERSCHMANN) 02 October 1990, see abstract.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or prior date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
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